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The Kinetics of Ornithine Decarboxylase Activity as a Function of Wounding in Guinea Pig Ear Epidermis

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Guinea pig ear stratum corneum was removed to increasing depths by 1, 3, 6 or 9 strippings with adhesive tape. Increasing ornithine decarboxylase (ODC) activity responses were observed in the epidermis 4.5 hr after wounding with 3, 6 or 9 tape strippings. The kinetics of this ODC response were investigated at intervals including, 2, 4.5, 12, 18, 24 and 72 hr after tape stripping. ODC activity was significantly elevated for 12 hr after 3 or 6 tape strippings and for at least 72 hr after 9 tape strippings. These effects were independent of hair plucking or depilation prior to wounding. This model is potentially useful for comparing the effects of chemical, mechanical and physical stimuli on ODC activity and subsequent polyamine synthesis.

The enzyme ornithine decarboxylase (ODC) converts ornithine into putrescine prior to cutaneous spermidine and spermine biosynthesis [1]. These polyamines then function in RNA, DNA and protein synthesis necessary for cutaneous cell proliferation. An increase in ODC activity has been observed in response to stimulation with chemical tumor promoting agents, such as 12-O-tetradecanoyl-phorbol-13-acetate (TPA) [2], ultraviolet irradiation [3], or mechanically induced wounding [4,5]. The increase in induced epidermal ODC activity is known to be dose dependent for chemical [2] and physical [6] stimuli, but this dose-dependence has not been determined for wounding by mechanical stimuli.

The present study determined that guinea pig ear epidermis is a suitable model for studying ODC activity, investigated the kinetics of the induced ODC activity response to mechanical removal of increasing amounts of its stratum corneum, and demonstrated that the induced ODC response to tape stripping is independent of hair plucking in this model.

MATERIALS AND METHODS

Materials

Male 300–400 g albino guinea pigs (Hartley strain) from Charles River laboratories were housed individually in stainless steel screen cages with *ad libitum* feed and water and a 12 hr light cycle from 6 AM to 6 PM.

DL-(1-^{14}C)ornithine hydrochloride (specific activity, 59.0 mCi/mM) was purchased from New England Nuclear, Boston, Massachusetts.

Industrial grade cloth tape* with a strong adhesive mass was cut into squares 1 cm on each side.

Removal of the Stratum Corneum

Squares of tape were successively applied to the dorsal surface of the undepilated guinea pig ears 1, 3, 6, or 9 times, removing surface stratum corneum each time the tape was stripped off. Each square of tape remained in place for at least 5 seconds before it was removed, to allow adhesion to the stratum corneum. Control data were taken from contralateral unwounded ears on 2 animals from each stripping condition at each time period.

The number of layers of stratum corneum remaining on the skin after 0, 1, 3, 6, or 9 strippings were counted to verify wound intensity differences. Frozen sections 6 μm thick were made on 4 ears in each stripping condition. Five sections from each ear were evaluated at 100 × magnification. After fixing sections for 5 min in 95% ethanol, a cover slip containing the frozen section was applied to a standard glass slide. Two drops of 0.25 N NaOH were applied to the edge of the cover slip. As the strong base penetrated the sample, stratum corneum cells swelled for easy visualization.

Hair Removal

To examine possible interactions between the observed increase in ODC activity and hair removal, which accompanied tape stripping, 2 techniques of hair removal were used with or without subsequent tape stripping (9 strips) on 8 animals per group. Hair was plucked from the dorsal surface of the ears 20 times per ear using a small diamond jaw hemostat, fitted with polyethylene tubing over one jaw. The second technique for hair removal was chemical depilation, accomplished with a gentle 10-min application of NEET† depilatory. Chemically depilated ears were thoroughly rinsed then dried for 15 min before tape stripping, in the group which received both chemical depilation and tape stripping.

Standard procedure for the ODC activity assay began 4.5 hr after treatment, when the ODC rsponse to 9 tape strippings was known to be at its maximum. For the ODC assay, the 8 ears per group were divided into 4 samples containing the epidermis from 2 ears each to permit adequate protein content of each sample. An untreated control group of 8 ears contained 2 contralateral untreated ears from each of the 4 treatment groups.

Assay of ODC Activity

The treated ears of 8 animals in each group were harvested 2, 4.5, 12, 18, 24 or 72 hr after wounding. Extra groups of 8 animals each were sampled 4.5, 24, 48 and 72 hr after wounding with 3 tape strippings, to confirm the effect of this wound, and 36 ± 4.5 hr after wounding with 9 tape strippings to explore that time span. Immediately before sampling, which took place between 9 AM and 1:30 PM, subjects were killed by intracardiac injection of T-61 Euthanasia Solution.‡

All tissues and extracts were maintained at 4°C except when specified. The dorsal epidermis was separated from the dermis after immersion in 55°C water for 30 seconds, by gentle teasing with a Teflon spatula. Each sample contained the pooled epidermis from 2 ears. Tissue from 8 ears was pooled to yield 4 samples per data point. Each sample was homogenized in 2 ml of 50 mM sodium phosphate buffer (pH 7.2) containing 0.1 mM pyridoxal phosphate, 2.5 mM dithiothreitol and 0.1 mM EDTA, then centrifuged at 30,000 × *g* for 30 min at 4°C to yield a clear soluble extract of ODC in the supernatant.

ODC activity in each extract was determined as described by O'Brien, Simsiman, and Boutwell [2], by measuring the release of $^{14}Co_2$ from DL-(1-^{14}C)ornithine. The extract was incubated for 10 min at 37°C in 5 ml test tubes with 101 μl of a medium of pyridoxal phosphate, 1 mM EDTA, 10 mM dithiothreitol, 50 mM sodium phosphate (pH 7.2) and 100 μl epidermal extract. Then 0.5 μCi DL-(1-^{14}C)-ornithine (59 mCi/mM) was added to the medium, bringing the final volume to 0.25 ml. The reaction proceeded for 60 min at 37°C until it was stopped by the addition of 1 ml of 2 M citric acid. The $^{14}CO_2$ resulting from this reaction was absorbed by 6.35 mm diameter absorbent discs[8] dipped in NCS

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Abbreviations:
ODC: ornithine decarboxylase
TPA: 12-O-tetradecanoyl-phorbol-13-acetate

* Permacel, U.S. Route 1, North Brunswick, New Jersey.

† Whitehall Laboratories, Inc., New York, New York.

‡ Animal Health Division, American Hoechst Corporation, Sommerville, New Jersey.

TABLE I. *Increase in ODC activity above that of contralateral unwounded ears as a function of increasing stratum corneum removal*

No. of tape strippings	Stratum Corneum Damage			
	1	3	6	9
No. of cell layers remaining in stratum corneum	10.3 ± 0.4	6.7 ± 0.9	2.6 ± 0.4	1.2 ± 0.1
Time after wounding	Increase in ODC Activity nM CO_2/hr/mg protein (Mean ± standard error of the mean)			
2 hr	—	0.02 ± 0.01	0.93 ± 0.03[a]	0.71 ± 0.18[a]
4.5 hr	0.02 ± 0.02	0.88 ± 0.29[a]	1.32 ± 0.20[a]	4.90 ± 1.33[a]
12 hr	0.01 ± 0.14	0.18 ± 0.03[a]	0.57 ± 0.08[a]	0.61 ± 0.09[a]
18 hr	0.00 ± 0.00	−0.03 ± 0.01[b]	0.01 ± 0.02	0.14 ± 0.04[a]
24 hr	−0.01 ± 0.00[b]	0.05 ± 0.02	0.02 ± 0.02	0.08 ± 0.01[a]
31.5 hr	—	—	—	0.16 ± 0.02[a]
36 hr	—	—	—	0.09 ± 0.01[a]
40.5 hr	—	—	—	0.14 ± 0.01[a]
48 hr	—	−0.01 ± 0.01[b]	—	—
74 hr	—	0.04 ± 0.00	0.05 ± 0.01	0.12 ± 0.02[a]

[a] ODC activity was significantly ($\alpha < 0.05$) higher than that observed in same-study control ears.
[b] Negative values indicate that ODC activity was lower than that of unwounded control ears, a result which may occur when group mean values lie within the error interval for the method.

tissue solubilizer[¶] and attached to the test tube stoppers. Incubation was continued for 60 min after the reaction was stopped to insure that all the $^{14}CO_2$ in the solution was absorbed by the discs. The absorbent discs were carefully removed and placed in scintillation vials containing 10 ml of scintillation cocktail comprised of 0.642% 2,5-diphenyl oxazole in toluene. Radioactivity was measured in a Beckman LS-250 liquid scintillation counter. Enzyme activities were determined in triplicate for each sample and corrected against blanks prepared by replacing the tissue extract with 100 μl of water.

Protein content was determined for each sample using an Abbott bichromatic analyser with a modification of the technique of Gornall et al [5]. Activity was expressed as nM CO_2 released in 1 hr/mg of protein in the epidermal sample.

RESULTS

Morphologically, the successively increasing number of tape strippings corresponded to removal of increasing numbers of layers of stratum corneum cells (Table I). The normal stratum corneum on either dorsal or ventral untreated guinea pig ears averaged 16 ± 6 layers of stratum corneum cells in thickness.

Phase contrast photomicrogaphs at 100 × magnification illustrate examples of each wound intensity in the Figure.

There was no change in ODC activity of untreated control ears throughout the experiment. ODC activity in these ears remained at 0.03 nM CO_2/hr/mg protein with a standard error of the mean of ± 0.02 for any group of 8 untreated control ears. All results are reported as increase in ODC activity above that observed in the control group for that time period.

ODC activity peaked 4.5 hr after wounding in all responding tape stripped groups (Table I). No substantial second peak in ODC activity was observed. There was also no increase in ODC activity in response to application and removal of a single square of tape. However, 3, 6 or 9 tape strippings produced successively higher peaks in ODC activity 4.5 hr after wounding and more prolonged intervals of elevated ODC activity as the number of tape strippings increased.

Neither plucking nor depilation stimulated an increase in epidermal ODC activity in this wound model (Table II). However, 9 tape strippings following either of these techniques of hair removal did elicit increased ODC activity 4.5 hr after treatment. That plucking removed nearly all the hairs from the dorsal surface of the ear was verified histologically.

Protein content of the samples averaged 0.13 ± 0.01 g/dl, with no significant differences observed among treatment groups.

[§] Schleicher & Schnell, Inc., Keene, New Hampshire.
[¶] Amersham/Searle, Inc., Arlington Heights, Illinois.

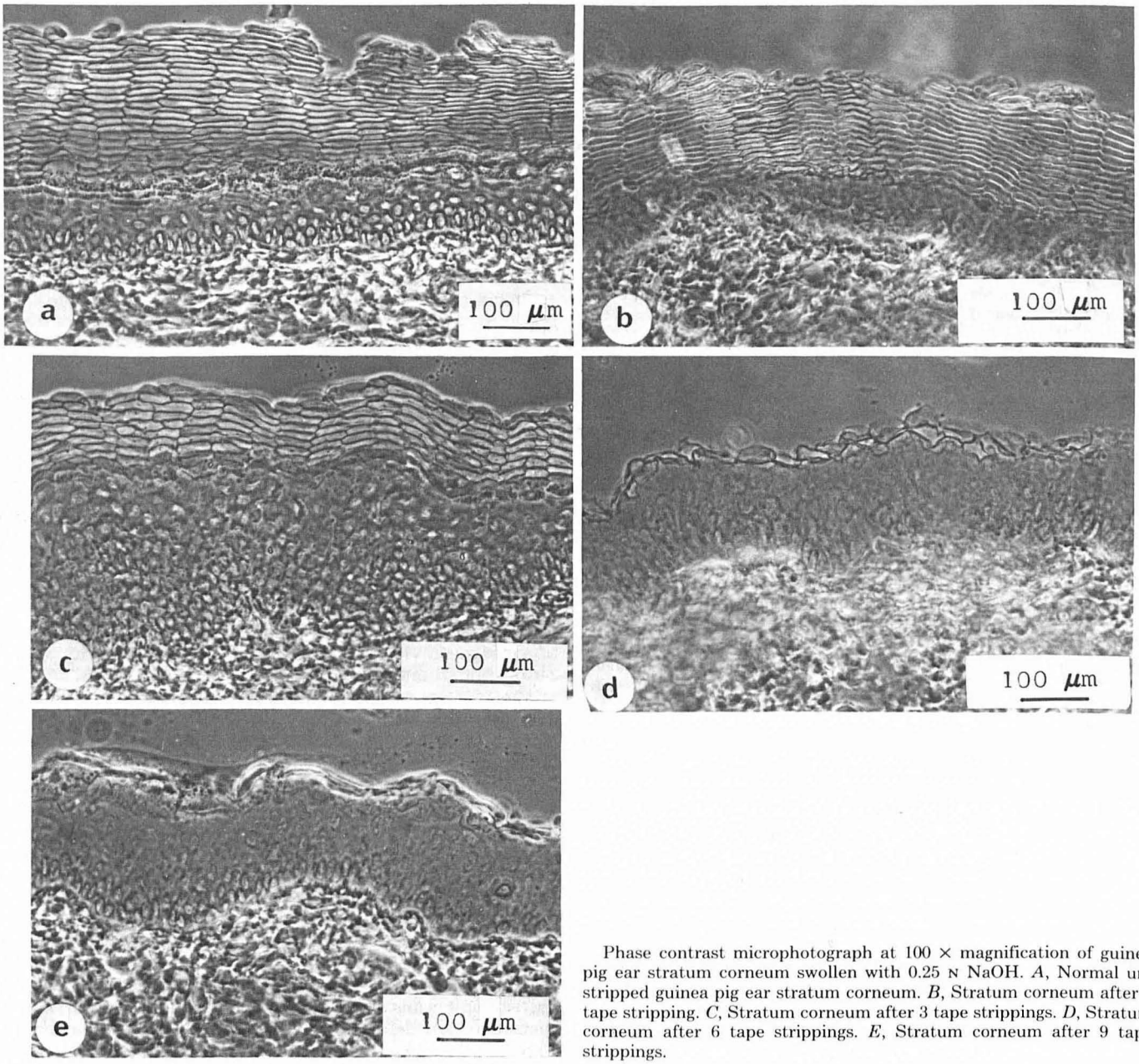
DISCUSSION

Because the tape stripped wound has a measureable area, this wound model can be regarded as a mechanical stimulus comparable to topical applications of chemical or physical stimuli. Mechanical stimulation by tape stripping induced a graded ODC activity response similar in kinetics to that observed in response to chemical stimulation with phorbol esters. Both these responses differ from that due to physical stimulation using ultraviolet light, which induces ODC activity peaking 24 hr after stimulation [3, 10], in hairless mice.

The kinetics of the proliferative response to vigorous tape stripping of guinea pig ears have been described in detail by Christophers [6]. An initial quiescent interval of 24 hr is followed by a sharp rise in proliferative activity by the epidermal basal cells, peaking 30 hr after wounding. A second similar peak in the proliferative response occurs 60 hr after wounding. ODC sampling times were chosen to cover similar intervals preceding both these peaks in proliferative activity, yet only a single peak in ODC activity was observed. Further research is needed to explore the relationship between ODC activity and epidermal proliferation in this wound model.

Morrison and Goldsmith [7] showed that hair plucking stimulated ODC activity in whole rat skin. Neither hair plucking nor gentle chemical depilation increased ODC activity in guinea pig ear epidermis. The difference in results may arise from species and site differences or from the different tissue sampled. The dorsal rat skin sampled by Morrison and Goldsmith has a much higher density of hair follicles than does the guinea pig ear. So hair plucking is more likely to disrupt perifollicular epidermis in dorsal rat skin than it is in the guinea pig ear. Moreover, hair plucking is known to stimulate hair growth in guinea pig skin [8]. Therefore one might expect plucking to increase ODC activity in whole skin samples containing follicular epidermis, such as those taken by Morrison and Goldsmith. However, the follicular epidermis extending into the dermis was not sampled in this study, which aimed at characterizing an effect of a skin surface stimulus.

The dose dependent ODC response observed here is consistent with recent results obtained by Clark-Lewis and Murray in albino Swiss mouse epidermis [9] showing that skin massage elicits no ODC response, while removal of the stratum corneum or incision induced marked increases in ODC activity. However, the peaks in ODC activity which Clark-Lewis and Murray observed at 24 and 72 hr did not occur in response to tape stripping. This difference in results could be due to species, hair growth cycle patterns in the Swiss mouse, wound model or circadian rhythm differences between experiments. Also, in their study no sample was taken between the time of wounding and 16 hr postwounding, preventing the observation of peaks in



Phase contrast microphotograph at 100 × magnification of guinea pig ear stratum corneum swollen with 0.25 N NaOH. *A*, Normal unstripped guinea pig ear stratum corneum. *B*, Stratum corneum after 1 tape stripping. *C*, Stratum corneum after 3 tape strippings. *D*, Stratum corneum after 6 tape strippings. *E*, Stratum corneum after 9 tape strippings.

TABLE II. *Increase in ODC activity above that seen in untreated control ear epidermis 4.5 hr after tape stripping with or without prior plucking or depilation*

Treatment (8 ears/group yielding 4 samples)	Increase above control ODC Activity nM CO_2/hr/mg protein (Mean ± standard error of the mean)
Plucked	0.07 ± 0.02
Depilated	0.05 ± 0.02
Plucked and tape stripped 9 times	1.45 ± 0.58[a]
Depilated and tape stripped 9 times	1.63 ± 0.12[a]
Tape stripped 9 times	1.81 ± 0.16[a]

[a] ODC activity was significantly higher than that of control ears.

ODC activity occurring between 0 and 16 hr after wounding.

Hair growth is asynchronous in guinea pigs, as it is in humans [7]. This independence of adjacent hair follicle growth cycles coupled with the relatively low density of the hair on the guinea pig recommend it as a model for the study of ODC activity and subsequent polyamine synthesis similar to that in human skin.

The present study shows not only that guinea pig ear epidermis is a suitable model for investigating the ODC activity response to skin surface stimuli, but that the graded response elicited by tape-stripping removal of the stratum corneum parallels that seen in CD-1 mouse skin [2] in response to skin surface chemical stimulation with TPA.

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Announcement

The 12th International Congress of the International Federation of the Society of Cosmetic Chemists will be held from September 13–17, 1982. Abstracts are due before November 30, 1981. For further information contact Société Française de Cosmétologie, 44, rue du 22 Septembre, 92400 Courbevoie, Paris, France.